1: J Clin Invest 1993 Jun;91(6):2850-6

Tissue factor pathway inhibitor reduces mortality from Escherichia coli septic shock.

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This study was designed to test the hypothesis that tissue factor pathway inhibitor (TFPI) plays a significant role in vivo in regulating coagulation that results from exposure of blood to tissue factor after vascular injury as in the case of gram negative sepsis. Highly purified recombinant TFPI (6 mg/kg) was administered either 30 min or 4 h after the start of a lethal intravenous Escherichia coli infusion in baboons. Early posttreatment of TFPI resulted in (a) permanent seven-day survivors (5/5) with significant improvement in quality of life, while the mean survival time for the controls (5/5) was 39.9 h (no survivors); and (b) significant attenuations of the coagulation response and various measures of cell injury, with significant reductions in pathology observed in E. coli sepsis target organs, including kidneys, adrenals, and lungs. TFPI administration did not affect the reduction in mean systemic arterial pressure, the increases in respiration and heart rate, or temperature changes associated with the bacterial infusion. TFPI treated E. coli infected baboons had significantly lower IL-6 levels than their phosphate buffered saline-treated controls, however tumor necrosis factor levels were similarly elevated in both groups. In contrast to the earlier 30-min treatment, the administration of TFPI at 4 h, i.e., 240 min, after the start of bacterial infusion resulted in prolongation of survival time, with 40% survival rate (2/5) and some attenuation of the coagulopathic response, especially in animals in which fibrinogen levels were above 10% of normal at the time of TFPI administration. Results provide evidence for the significance of tissue factor and tissue factor pathway inhibitor in bacterial sepsis, and suggest a role for blood coagulation in the regulation of the inflammatory response.

PMID: 8514893 [PubMed - indexed for MEDLINE]

1: Gene 1993 Dec 27;137(1):25-31

The discovery and characterization of a novel nucleotide-based thrombin inhibitor.

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Thrombin is a serine protease that plays a pivotal role in thrombosis and hemostasis, and is a major target for anticoagulation and cardiovascular disease therapy. Using a novel in vitro selection/amplification technique, we have identified a new class of thrombin inhibitors based on single-stranded DNA (ssDNA) oligodeoxyribonucleotides (oligos). These thrombin inhibitors are the first example of the use of this technique to obtain ssDNA oligos that bind a target protein that does not interact physiologically with nucleic acid. Here, we review how iterative selection and amplification were used to identify short ssDNA sequences that bind and inhibit thrombin (Bock et al., Nature 355 (1992) 564-566), and the tertiary structure of one aptamer sequence (Wang et al., Biochemistry 32 (1993) 1899-1904). Results from in vitro and in vivo studies are also summarized (Griffin et al., Blood 81 (1993) 3271-3276). The discovery of a new class of thrombin inhibitors using this technology demonstrates the power of this new approach for rapid drug discovery and development.

PMID: 8282198 [PubMed - indexed for MEDLINE]

Autoantibodies directed against the epidermal growth factor-like domains of thrombomodulin inhibit protein C activation in vitro.

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No consensus has been obtained about the question whether autoantibodies, in particular antiphospholipid antibodies (aPL), may cause thrombosis by inhibiting thrombomodulin (TM) mediated protein C activation. In order to clarify the mechanism by which autoantibodies inhibit TM-mediated protein C activation, we have screened 12 patients with autoimmune diseases for the presence of circulating autoantibodies inhibiting TM function. In a cross-sectional study we found that IgG fractions from two patients (who were aPL negative) inhibited TM mediated protein C activation in an assay system using purified components. A longitudinal study of six patients with a history of thrombosis of which two were aPL positive showed that all had at some time circulating antibodies inhibiting TM function, suggesting that the presence of these antibodies is transient. Three different TMs were used to identify the epitope of the antithrombomodulin antibodies (aTM): rabbit TM, which contains the entire TM molecule; Solulin, which contains the extracellular part of TM, and rEGF-TM, which contains the six epidermal growth factor (EGF) domains of TM. We showed that the aTM inhibited protein C activation mediated by all three TMs, indicating that the aTM are directed against the region containing the EGF domains. When TM was incorporated in phospholipid vesicles, no inhibition by these aTM could be demonstrated. In addition, protein C activation mediated by cultured endothelial cells (EC) could not be inhibited by aTM. The lack of inhibition of TM in phospholipid vesicles and EC-TM by a TM suggests that aTM only inhibit soluble TM. In conclusion, we demonstrated the transient presence of circulating autoantibodies directed against the region of TM containing the EGF domains in SLE patients with a history of thrombotic complications. We postulate that the presence of antibodies to soluble TM may be, in addition to aPL, a risk factor for the occurrence of thrombosis in patients with autoimmune diseases.

PMID: 7522520 [PubMed - indexed for MEDLINE]

1: Thromb Haemost 1993 Sep 1;70(3):448-53

The significance of TFPI in clotting assays--comparison and combination with other anticoagulants.

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The anticoagulant activities of Tissue Factor Pathway Inhibitor (TFPI), heparin and hirudin were compared in intrinsic (APTT) and extrinsic (PT) activated clotting assays. In contrast to the thrombin inhibitor hirudin, heparin was 10 fold more potent in the APTT assay than in the PT assay, indicating that inhibition of intrinsic activation is important for the anticoagulant activity of heparin as measured in an APTT assay. TFPI was most potent in the PT assay and the effect of TFPI was most pronounced in the presence of other anticoagulants (heparin and hirudin). The activities of the two natural anticoagulants antithrombin III (ATIII) and TFPI were compared in a PT assay with very dilute tissue factor. In this assay system TFPI in normal plasma affected the clotting time more than ATIII in the plasma. However, when heparin was added ATIII was the major anticoagulant, but profound prolongation of the clotting time was only seen when TFPI was also added. In an ATIII deficient plasma heparin did not augment the effect of TFPI, showing that the increased effect of TFPI in the presence of heparin is dependent on the anticoagulant activity of ATIII/heparin. The effect of TFPI at prolonged clotting times was also illustrated by the significant effect of blocking TFPI in the plasma from warfarin-treated patients. Thus TFPI is a major anticoagulant in normal plasma and the effect of TFPI is especially seen at prolonged clotting times.

PMID: 8259547 [PubMed - indexed for MEDLINE]

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